CASE 60116P1

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Melissa Hardy	Melissa Hardy	4/21/2005
Type or print name	Signature	Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Glenn R. Bowers CONFIRMATION NO: 2458

APPLICATION NO: 10/007,038

FILED: December 4, 2001

FOR: Soybean Cultivar S52-U3

DECLARATION OF JOHN ARBUCKLE

I, John A. Arbuckle, declare and state:

That I am a citizen of the United States and I reside at 12350 124th Ct. E. Northfield, Minnesota. That I graduated in 1992 from Illinois State University located in Normal, Illinois with a Bachelor of Science Degree in Biology.

That since 1992 I have been working in the field of agriculture;

My molecular genetics professional experience is as follows:

8/92 - 8/96	**Research Associate / Team Leader** *Research Specialists - Applied Genetic Marker Laboratory* Pioneer Hi-Bred International, Inc.
8/96 – 5/98	**Senior Research Associate** *Genome Research Group – Forward/Reverse Genetics Library Construction* Pioneer Hi-Bred International, Inc.
5/98 – 7/99	**Research Manager** *Analytical Biochemistry – Maize Molecular Markers* Pioneer Hi-Bred International, Inc.
7/99 – present	**Scientist III / Molecular Marker Laboratory Manager** *Crop Genetics Research – Seeds* Syngenta

I have supervised the experiments described below;

Our USST Molecular Maker Laboratory, located in Stanton, Minnesota, conducted a routine genetic analysis of Soybean Cultivar S52-U3 and the Monsanto soybean cultivar referred to as 9524889614923;

As background, genetic fingerprinting is a technique to distinguish between individuals of the same species using only samples of their DNA. Two individuals of the same species will have the vast majority of their DNA sequence in common. There are a number of different technologies for obtaining a genetic fingerprint. The original method was based on minisatellites. Minisatellites are regions where particular DNA sequences are repeated many times, and the number of times varies enormously between individuals. However, minisatellites have now been almost entirely replaced by microsatellites (aka Simple sequence Repeats – SSRs) which are similar except that they detect very short repeating sequences and the detection system is based on the Polymerase Chain Reaction (PCR) which means we can determine a fingerprint from very small or very old samples of blood or tissue (even a single hair). Genetic fingerprinting exploits these highly variable microsatellites. Two unrelated individuals will be likely to have different numbers of microsatellites at a given locus (polymorphisms). By using PCR to detect the number of repeats at several loci, it is possible to establish a match that is extremely unlikely to have arisen by coincidence. Conversely, the degree of relatedness between two individuals can be inferred from the degree of polymorphism.

SSR markers can be employed in the development of unique allelic profiles for establishing individual identity. Distinctive profiles can be readily generated by defining the allelic constitution of individuals at relatively few loci, each of which is multi-allelic. Such a system is open-ended in that additional loci can be added if those already in use are inadequate to produce a unique profile for all individuals.

Several peer-reviewed publications demonstrate SSR markers can be used to differentiate between soybean genotypes. One such manuscript is by Song, et al. (Song *et al.* 1999, *A selected set of trinucleotide simple sequence repeat markers for soybean cultivar identification,* Plant Varieties and Seeds 12:207-220). In summary, the authors examined many SSR loci in order to select a discriminating set for DNA profiling of soybean varieties. Only SSR loci with allele size ranges that showed no overlap in size over a series of analyses and in which adjacent alleles differed by at least three basepairs were maintained for further statistical analysis via a clustering procedure. Cluster analysis was performed on 30 SSR loci and resulted in the identification of a subset of 13 loci, from 12 different linkage groups, that easily produced unique SSR allele size profiles for each of a set of 66 elite North American soybean cultivars. This set of 13 loci was used to characterise four independent sets of elite cultivars that were selected based upon identical maturity, morphological, and pigmentation traits. Based upon these analyses, all cultivars could be distinguished using the set of 13 selected SSR loci. This set of loci was proposed by the authors as a standard set for use in DNA profiling of soybean cultivars for purposes of obtaining PVP.

We compared 9524889614923 with the soybean cultivar S52-U3 using a set of simple sequence repeat markers proven to provide reproducible data in our laboratory that are distributed across each of the 20 soybean linkage groups and includes 4 of the 13 described by Song et al. We found polymorphism for 22 of the 31 SSR markers. Our genetic data establishes a Neis score of 0.290.

$$\text{Neis} = \frac{\text{the number of markers that match (monomorphic)}}{\text{total number of markers}}$$

Thus, our genetic testing shows that the soybean variety S52-U3 is only 29% related to the soybean cultivar 9524889614923.

That the undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or

imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

April 21, 2005
Date

John A. Arbuckle